Review

Influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods

Ryszard Amarowicz[1], Reinhold Carle[2], Gerhard Dongowski[3], Alessandra Durazzo[4], Rudolf Galensa[5], Dietmar Kammerer[2], Guiseppe Maiani[4] and Mariusz K. Piskula[1]

[1] Institute of Animal Reproduction and Food Research, Polish Academy of Science, Olsztyn, Poland
[2] Institute of Food Technology, University of Hohenheim, Stuttgart, Germany
[3] German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany
[4] National Research Institute for Food and Nutrition, Roma, Italy
[5] Institute of Nutrition and Food Sciences, University of Bonn, Bonn, Germany

The review is based on the evaluation of electronically collated data published between 2002 to June 2006. It is based on 325 references dealing with the following subclasses of phenolic compounds: hydroxycinnamic and hydroxybenzoic acids, chalcones, flavanones, flavones, flavonols, monomeric flavanols and anthocyanins. Only publications dealing directly with the effects of storage and postharvest processing on the phenolic acid and flavonoid contents of foods were considered. The expectation that the structural diversity even within each subgroup, and the number of different procedures and of different parameters would make finding homogenous tendencies unlikely, has, in most instances, been confirmed. By adding a database Excel table combined with a focused and unified evaluation, specific additional information was rendered accessible and concise. It holds true for most of the subclasses in question that the effect of storage and food processing on the polyphenol content is negligible in comparison to the differences between different varieties of plants. Variety dependence must always be considered, for all classes of compounds.

Keywords: Flavonoids / Food processing / Food storage / Phenolic acids / Postharvest processing

Received: Novmber 27, 2007; revised: April 8, 2008; accepted: May 5, 2008

1 Introduction and data evaluation

The following review is based on the evaluation of electronically collated data published on phenolic acids and flavonoids between 2002 and June 2006. It contains 325 references dealing with the following subclasses of phenolic compounds: hydroxycinnamic and hydroxybenzoic acids, chalcones, flavanones, flavones, flavonols, monomeric flavanols and anthocyanins.

The aim of this work was to gain data on the content of the phenolic acids and flavonoids which could then be used to provide first indications on their bioavailability in the analysed foods. As there are already a multitude of publications about these substances, including some relatively new reviews, it was decided to focus on the available literature of publications dealing directly with the effects of storage and postharvest processing on the phenolic acid and flavonoid content of foods. More detailed information about the documented influence of postharvest processing and storage on the bioavailability of flavonoids and phenolic acids in foodstuffs will be published elsewhere [1].

The selected publications have been evaluated according to a given pattern and the acquired data compiled into an extensive Excel table (see Supporting Information) with the following column titles:

'Food source' (includes all mentioned foods in the cited publication), 'Substance classes', 'Individual compounds' (includes the names of all mentioned compounds in the cited publication), 'Postharvest action', 'Aspects of storage', 'Aspects of food processing' (these three columns contain the treatments or procedures investigated, *e.g.* storage conditions, freezing, irradiation, bread making, fermentation and so on), 'Aspects of content' (provides details about changes in content produced by processing proce-

Correspondence: Professor Rudolf Galensa, Department of Food Chemistry, Institute of Nutrition and Food Sciences (IEL), University of Bonn, Endenicher Allee 11-13, 53115 Bonn, Germany
E-mail: galensa@uni-bonn.de
Fax: +49-228-733757

Abbreviation: CA, controlled atmosphere; **FW,** fresh weight; **MA,** modified atmosphere; **MCP,** 1-methylcyclopropene; **PAL,** phenylalanine ammonia lyase; **PPO,** polyphenoloxidase

dures), 'Aspects of bioavailability' (gives brief information about whether there are any aspects mentioned; this is important for the second part of the publication which deals with bioavailability [1]), 'Complete title of the application', 'Authors', and 'References'.

This Excel table is available as Supporting Information. In this electronic form, individual searches (*e.g.* for individual compounds or treatments) can be performed using normal Excel functions.

Selected typical publications from the Excel (without the bibliographic data) are summarized in Table 1.

In the following sections, the most important data according to the different compound classes are discussed in detail with five identical subtitles for each class:

'Biosynthesis' (includes brief information about the position of the class of compounds in the pathway and/or individual specialities), 'Food sources' (the list of the investigated foods combined with the relevant numbers of the Excel table, see Supporting Information), 'Aspects of postharvest processing and storage procedures' (this list gives an enumeration of all the different measures or procedures investigated, combined with the corresponding numbers in the Excel table, see Supporting Information), 'Structures of individual compounds, content and changes in content in foods' (this section is the most important part; it includes a short evaluation of interesting results for some substances and how their contents changes in foods under the described procedures), and 'Summary' of the class of compounds.

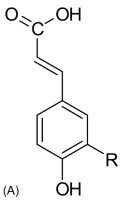
In some references, the effects of postharvest processing and storage on the content of total polyphenols or general effects of phenolic compounds are given [8, 10, 24 – 38].

2 Phenolic acids (hydroxycinnamic acids, hydroxybenzoic acids)

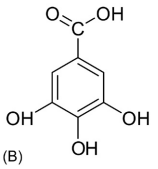
The structure of hydroxycinnamic and hydroxybenzoic acids is given in Fig. 1.

2.1 Biosynthesis

The phenolic acids present in plant raw material and food of plant origin are derivatives of two phenolic compounds – benzoic and cinnamic acids. The basic pathway of synthesis of phenolic acids in plants leads from sugars through to aromatic amino acids – phenylalanine, and, in some rare cases, tyrosine. Deamination of amino acids to the appropriate phenolic acids occurs as follows: *trans*-cinnamic acid from phenylalanine and *p*-hydroxycinnamic acid from tyrosine are catalysed by phenylalanine ammonia lyase (PAL). Derivative of cinnamic acid are generated during the successive methylation and hydroxylation of cinnamic acid catalysed by cinnamic acid hydroxylase (C4H). Derivatives of benzoic acid can be generated from dihydroshikimic acid or *p*-coumaric acid through *p*-coumaroyl/CoA. Synthesis of



Hydroxycinnamic acids
p-coumaric acid: R = H
caffeic acid: R = OH



Hydroxybenzoic acids
gallic acid

Figure 1. Structure of hydroxycinnamic acids (A) and hydroxybenzoic acids (B). Figure has kindly been provided by Rainer Cermak [1].

phenolic acid thioesters occurs with the aid of ligase/*p*-coumaric acid/CoA (4CL).

2.2 Food sources

The following list presents the food sources of phenolic acids and the papers in which they were discussed:

Apples and apple juice [16, 39 – 41], cherries [23, 42 – 44], pears [45, 46], must and wine [17, 20, 47 – 54], sherry vinegar [55], sherry wine [56], winemaking waste solids [57], blueberries and blueberry juice [14, 58 – 62], raspberries [4], strawberries [63, 64], bayberries (*Myrica rubra*) [65], orange peel [66], mangos [67], passion fruit juice [68], dates [69], broccoli [70, 71], lettuce [3], tomatoes [72 – 74], potatoes [75, 76], jicama [77], carrots [78], asparagus [79, 80], leavy vegetables [81], legumes [22, 53, 82], olives and olive oil [83 – 85], rye [86], oat [87], sorghum [88], wheat bran [89, 90], cereal brans [91], barley [92], rice [93], sesame [94], sea buckthorn [95], mustard seeds [96], coffee beans [97], soybeans [98, 99], artichokes [100], yams (*Discorea* spp.) [101], roselles (*Hibiscus sabdariffa* L.) [102], and *Argania spinosa* oil and press cake [103].

2.3 Aspects of postharvest processing and storage procedures

The following aspects are mentioned in the papers:

Storage under different conditions [3, 14, 16, 20, 23, 40, 41 – 43, 46, 49, 53, 63, 67, 68, 70, 74, 75, 77 – 80, 83, 91, 100, 102], washing treatment [78, 104], blanching [59, 65, 101], drying [102], sun-drying [37, 45], treatment with a growth regulator [23, 41], alkaline and acid hydrolysis [90],

Table 1. Influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods

Food source	Substance classes	Individual substances	Postharvest action	Aspects of storage	Aspects of food processing	Aspects of content	Aspects of bioavailability	References
Grape juices, wine	Anthocyanines, flavonols, ellagic acid	3,5-Diglucosides of delphinidin, cyanidin, petunidin, pelargonidin, peonidin, malvidin, myricetin quercetin, kaempferol, ellagic acid	No	60 days	Juices and wines were produced by hot- and cold-pressed techniques; wine was produced following on-hull fermentation for 3, 5 and 7 days	After storage, wines had lower concentration of individual polyphenolic compounds; processing methods were important factors influencing flavonoids	No	[2]
Brocooli, lettuce	Total phenolics	No	Storage in argon, helium and nitrogen atmosphere containing 2% oxygen	7 and 9 days	No	The content of total phenolics was reduced in relation to the control sample (stored at air)	No	[3]
Red raspberries	Total phenolics, anthocyanins	Cyanidin-3-sophoroside, cyanidin-3-(2G-glucosylrutinoside), cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-sophoroside, pelargonidin-3-glucose-rutinoside	Freezing	Storage at 4°C for 3 days and then at 18°C for 24 h	No	Anthocyanin levels were unaffected while vitamin C levels declined and those of elligitannins increased; no effect on the antioxidant capacity	No	[4]
Oranges	Vitamin C, phenolics, flavones, flavanones, hydroxycinnamates	L-Ascorbic acid and L-dehydroascorbic acid, caffeic acid derivatives, vicenin 2, narirutin	Squeezing, mild pasteurization, standard pasteurization, concentration, freezing	No	Squeezing, mild pasteurization, standard pasteurization, concentration and freezing	Freezing process caused a decrease in phenolics; pasteurization increased vitamin C content	Yes	[5]
Rooibos	Flavonoids, chalcone	Aspalatin, nothofagin	No	No	Fermentation	Loss of both dihydrochalcons	No	[6]
Grape juice	Procyanidins, catechins, flavones	Flavan-3-ols, (+)-catechin, (-)-epicatechin	Pressing	No	Pressing, pasteurization	Pasteurization increased the concentration of catechins in cold-pressed juices, but it decreased concentrations in hot-pressed juices; concentration of most procyanidins was increased by pasteurization	No	[7]
Marionberries, strawberries, corn	Phenolics, flavonols	Ascorbic acid	Freezing, freeze-drying, air-drying		Freezing, freeze-drying, air-drying	Freeze-drying preserved higher levels of total phenolics in comparison with air-drying	No	[8]
Orange juice	Flavanones	Naringenin glycosides, hesperidin glycosides	No	No	Freshly squeezed juice, traditional pasteurization, short-time pasterization, freezed juice	Highest flavanone content in traditional pasteurized juice; lowest flavanone content in freezed juice	No	[9]
Tomato products (pulp, puree, paste)	Total phenolics	Rutin	No	Yes	3 months at 30, 40 and 50°C	Decrease in total phenolics at >40°C	No	[10]
Apples	Flavanones, flavanols	Luteolinflavan, luteolinflavan-5-glucoside, eriodictyol-7-glucoside, 6‴-O-trans-p-coumaroyleriodictyol-3″-glucoside	Freezing: the leaves were frozen in liquid nitrogen	No	Lyophilizing: apple leaves were lyophilized	The content of phenylpropanoids was influenced by prohexadione	Yes	[11]

Table 1. Continued

Food source	Substance classes	Individual substances	Postharvest action	Aspects of storage	Aspects of food processing	Aspects of content	Aspects of bioavailability	References
Plums	Flavonols, anthocyanidins	Rutin, quercetin-3-galactoside, quercetin-3-glucoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, peonidin-3-glucoside	Refrigerating: plums were stored at 2 – 5°C	No	Cutting, lyophilizing, freezing: plums were cut in half and the pits removed; pitted plums were frozen and lyophilized, and then dried samples were ground to powder and stored at −20°C	Determination of flavonoids of different variety of plums: rutin was the most predominant flavonol	No	[12]
Apples, cherries, strawberries, blackberries, grapes, apple juice	Hydroxybenzoic acids, hydroxycinnamic acids, flavanols, flavonols, anthocyanidins, dihydrochalcones	Gallic acid, p-coumaric acid, chlorogenic acid, (+)-catechin, (−)-epicatechin, procyanidin B1, procyanidin B2, kaempferol, quercetin, phlotetin, phloredzin, pelargonidin, cyaniding	The fruits were purchased from a local supermarket	No	Peeling (for apples), depitting (for cherries and grapes)	Determination of flavonoid concentration in different parts of each fruit	No	[13]
Blueberries	Anthocyanidins, flavonols, hydroxycinnamic acids	Chlorogenic acid, myricetin-3-arabinoside, quercetin-3-galactoside, quercetin-3-arabinoside, delphinidin-3-glucoside, delphinidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-galactoside, petunidin-3-glucoside, petunidin-3-galactoside, malvidin-3-galactoside, malvidin-3-glucoside, malvidin-3-arabinoside	Blueberries were sorted to eliminate damaged, shriveled, and unriped fruit, and selected for uniform size and colour	Freshly harvested blueberries were placed in jars, ventilated continuously with air or with 40, 60, 80 or 100% O_2 at 5°C for up to 35 days	No	Changes of flavonoids in blueberry during storage in air or high-O_2 atmospheres	No	[14]
Grapes	Flavanols, anthocyanins	Delphinidin, cyanidin, petunidin, peonidin, malvidin-3-glucoside, malvidin-acetyl-3-glucosides, malvidin coumaroyl-3-glucosides, malvidin caffeoyl-3-glucosides, vitisin B, catechin, epicatechin (dimers, trimers, tetramers), coumaric acid derivatives, galloylated derivatives	Selection: after being sorted to remove damaged grapes, that were destemmed and transferred to the vats for controlled fermentation at a temperature of between 25 and 28°C with the addition of a small amount of SO_2; fermentation	No	Fermentation	Determination of flavonoids in according to different period of grape-harvest: the characteristics and the composition of grapes harvested later than the usual time are quite beneficial to obtaining quality aged wines	No	[15]
Apples	Polyphenols, flavonoids, flavones	Cholorogenic acid, phloridzin, catechins	Storage	Storage for 4 month	No	During storage, concentration of catechin and phloridzin increased	Yes	[16]

Table 1. Continued

Food source	Substance classes	Individual substances	Postharvest action	Aspects of storage	Aspects of food processing	Aspects of content	Aspects of bioavailability	References
Sherry wine	Hydroxybenzoic acids, hydroxycinnamic acids, flavanols	Gallic acid, syringic acid, cartaric acid, 2-S-glutathionyl caftaric acid, cis p-coutaric acid, trans p-coutaric acid, fertaric acid, caffeic acid, trans p-coumaric acid, ferulic acid, procyanidin B1, catechin, procyanidin B2, epicatechin	Different degrees of destemming (0, 25, 50 and 75%), fermentation of musts	Sampling after 0, 2, 4, 6 and 9 days	Fermentation	Increase of some compounds during alcoholic fermentation	No	[17]
Hop	Chalcone, flavone	Xanthohumol, 8- and 6-prenyl-naringenin, isoxanthohumol	Extraction with CO_2 and alcohols	No	Extraction	Improved extractability of chalcones and flavones	No	[18]
Wheat	Phenolics, flavonoids, ferulic acid, carotenoids	Catechin, lutein, zeaxanthin, β-cryptoxanthin	Milling	No	Milling (endosperm and bran/germ fractions)	Different milled fractions of wheat have different profiles of both hydrophilic and lipophilic phytochemicals	Yes	[19]
Red wine	Polyphenols	Gallic acid	No	Storage at 25 and 37°C	No	The older wines had a lower antioxidant ability	No	[20]
Grapefruit juice and pulp	Flavanones, furanocoumarins	Bergamottin, 6′,7′-dihydroxybergamottin, 6′,7′-epoxybergamottin, 7-geranyloxycoumarin	Extracting juice	No	Freshly extracted juice [raw finished juice (~5% fine pulp), centrifugal retentate (~35% fine pulp), centrifuged supernatant (<1% pulp), and coarse finisher pulp]	The centrifugal retentate had the highest furanocoumarin content	Yes	[21]
Beans	Flavanols, hydroxybenzoic acids, hydroxycinnamic acids	Kaempferol, quercetin, p-hydroxybenzoic acid, vanillic acid, p-coumaric acid, ferulic acid (daidzein, genistein, coumestrol)	Germination	No	Cooking	Decrease during cooking; increase in germination	No	[22]
Sweet cherries	Hydroxycinnamic acids, anthocyanins	Cyanidin, pelargonidin, peonidin, neochlorogenic, p-coumarylquinic acids	Treatment with MCP	Cold storage	No	Alteration not significant	No	[23]

juice centrifugation [65], flash release process [54], cowinemaking and wine aging [52, 56, 105], grape destemming [17], fermentation [44, 57], germination [22, 82, 93], extraction [60, 84, 85, 89], thermal stability [44], bread making [86], cooking [22], roasting [97], stir-baked processing [96], steaming [87], thermal treatment [50, 58, 68, 81], SO_2 treatment [106], break-process [72], freezing [4, 71], freeze-drying [64], maceration [47, 48, 51], enzymatic treatment [39, 60], malting [92], and peeling and cutting [76].

The observed effects of some of these will be discussed with reference to their application in given foodstuffs in the following sections.

2.4 Structures of individual compounds, content and changes in content in foods

The storage of seven commercial apple juices for 11 months resulted in a decrease in phenolic acids from 5 to 21% [40]. Apples (Annurca variety) showed a marked increase in chlorogenic acid during storage: 101 mg/kg fresh weight (FW) after harvest, 131 mg/kg FW after 3 months and 144 mg/kg FW after 4 months [16]. In raw juices obtained from apples using straight pressing, the level of chlorogenic acid was reduced to about 50% when compared to that in fresh apples [39]. The chlorogenic acid biosynthesis of apples harvested early or at optimum maturity was greatly inhibited by a growth regulator during a

120-day storage period and 1 wk shelf-life [41]. Sun-drying modified the content of chlorogenic and *p*-coumaric acids in the methanol extract obtained from Portuguese pears (*Pyrus communis* L. var. S. Bartolomeu) [45].

The storage period (6 or 30 days) induced some variation in the phenolic acid content of cherries, although the final tendency was a reduction in these levels after storage at 1–2°C and an increase in cherries at 15 ± 5°C [42]. The changes in hydroxycinnamates among the four cherry cultivars during cold storage (2°C for 30 days) were not consistent [43]. Several phenolic acids (protocatechuic, chlorogenic, caffeic and *p*-coumaric acids) were found during production of permez made from the concentrated juice of cherry laurel varieties [44]. After the storage of sweet cherries treated with a growth regulator, the content of chlorogenic acid decreased from 427 to 334 mg/kg FW [23].

The heat and SO_2 treatments of blueberries did not change the content of total phenolic acids (the sum of *p*-hydroxybenzoic, vanillic, chlorogenic, caffeic, syringic, ferulic and *o*-coumaric acids) in pressed juice, clarified juice, pasteurized juice and concentrate [58]. No significant differences in the content of chlorogenic acid in highbush blueberries were observed during storage at high-oxygen atmospheres (at 5°C for 35 days; 40, 60, 80 and 100% O_2) [14]. The total cinnamate content of highbush blueberry juices made from blanched material was 348 mg/kg juice and from nonblanched material 26.0 mg/100 g [59]. Enzyme treatment of the blueberry processing waste had little effect on total phenolic recovery. Cinnamic acid derivatives (chlorogenic, caffeic and syringic acids) were found in the skins and seeds [60]. The fermentation of the lowbush blueberry by a novel bacterium from the fruit microflora resulted in the production of gallic acid [61].

The content of hydroxybenzoic acids increased (50–150%) in the centrifuged juices of bayberries, after the primary steps of processing (crushing, depectinization, centrifugation, pasterization and blanching) [65]. Storage (4°C for 72 h) of strawberries reduced the content of *p*-coumaric and chlorogenic acids from 74 to 58 μmol/100 g FW and from 440 to 320 μmol/kg FW, respectively [63]. The content of cinnamic acid derivatives in the flesh of strawberries expressed as chlorogenic acid was not affected by freeze-drying [64]. A decrease in the content of free *p*-coumaric acid and an increase in that of conjugated *p*-coumaric acid were observed in frozen red raspberries [4].

The extraction efficiency of hydroxycinnamic acids from olive mill waste was highest when 50% v/v aqueous solutions of ethanol, propanol, ACN and acetone were used [85]. The two-phase decanter method preserved more of the phenolic content than the three-phase method by which the oil, water and husk is separated from the olive paste [84]. Far-infrared irradiation may be able to cleave covalent bonds and liberate phenolic acids from the repeating polymers present in sesame seeds [94]. Storage temperature was the major factor contributing to the changes in gallic acid content in mangos. Free gallic acid was unaffected by hot water treatment (for 60 min at 50°C) [67]. Sun-drying significantly increased the concentration of free and bound phenolic acids (gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic and *o*-coumaric acids) of three varieties of dates [69].

During postharvest storage, asparagus experienced a general increase in ferulic acid monomers and dimers that affected every section of both green and white spears [80]. Storage of asparagus spears (21°C for 3 days) resulted in a considerable increase in phenolic acids (at least three-fold) in each section, particularly in the lower section, and was accompanied by an increase in the proportion of diferulic acid moieties in the mid and lower sections [79]. After 3 wk of postharvest storage, the content of chlorogenic acid in cherry tomatoes was found to be 35% higher than that in the freshly sampled ones [74]. The super cold break-process used for tomato sauce at 65°C under vacuum produced an increase of about 30% in the content of caffeic, *p*-coumaric and ferulic acids [72]. The content of phenolic acids (the sum of chlorogenic and coumaroylquinic acids) increased throughout the storage of carrots under aerobic conditions [78].

Hand peeling and cutting into strips with a manual potato cutter at room temperature, washing with running water and followed by refrigerated storage (1–6 days) caused a 2.4–8.6-fold increase in the chlorogenic acid content of potatoes [76]. Storage in controlled atmospheres (CAs, 2% O_2) reduced the accumulation of phenolic compounds by about 35% in both fresh-cut crisphead (Iceberg) lettuce and in the green leaf variety [3]. After cold storage, broccoli lost about 75% of its caffeoyl-quinic derivatives and 40–50% of its sinapic acid and feruloyl derivatives [70].

Destemming of grapes prior to pressing changed the phenolic acid content in Polomino fino sherry samples after 2 days of inoculation and at the end of fermentation but no relationship was observed between degree of destemming and changes in phenolic acid content [17]. The must of grapes treated with the flash release process was characterized by higher amounts of hydroxycinnamic acids [54]. After aging in French oak barrels, wine showed a significant increase in gallic and syringic acids content. This is not surprising as gallic and syringic acid are wood constituents. The ferulic acid content decreased in all wines [52]. The use of macerating enzymes and two enological tannins for the making of Monastrella wines had no effect on the content of gallic, syringic, protocatechuic, *m*-hydroxybenzoic, vanillic, coumaric, caftaric and ferulic acids but reduced the content of caffeic acid and modified the content of coutaric acid at the bottling stage [51]. The phenolic compound in the wines diminished with storage time, with the exception of caffeic, ferulic and *p*-coumaric acids. Hydrolysis is mainly responsible for the increase in free phenolic acids [53]. No differences in hydroxycinnamic acid derivative (*trans*-caffeoyltartaric and *trans*-*p*-coumar-

oyltartaric) content between conventional and ecological red and white wines were observed [49]. In blackcurrant wines, the polyphenol extraction was highest after maceration and pectinolysis. In cherry wines, the highest amount of these compounds was extracted after pectinolysis and pasteurization [47]. The concentration of gallic acid increased in samples of sherry vinegars aged in American oak butts for 360 days. For protocatechuic, *p*-hydroxybenzoic and vanillic acids, there was great variation of the contents [105].

During the soaking period prior to germination, the content of phenolic acids in legume seeds (beans, peas and lentils) decreased. During germination, the increase in the phenolic acids showed different behaviour patterns in those seeds [82]. Washing (three times with sterile distilled water under aseptic condition) and drying (at 55°C for 24 h) slightly modified the phenolic acid composition of cowpea seeds [104]. The germinated bean seeds exhibited lower *p*-hydroxybenzoic acid content, higher vanillic acid and *p*-coumaric acid content than raw bean seeds, while the ferulic acid content were similar [22]. The phenolic acid content of the soybean yellow sprouts produced under dark conditions and green sprouts grown in green and yellow boxes were different [99].

The application of ‚activated Germination Malting' caused a limited increase in the concentrations of ferulic and *p*-coumaric acids in malt, whereas a higher temperature during malting resulted in approx. two-fold higher concentrations of both free phenolic acids in kilned malt. The use of steeping water of pH 5.2 instead of 7.4 resulted in a significant increase in the content of free ferulic and *p*-coumaric acids in malt, which could possibly lead to an increase in the content of these phenolic acids in beer [92]. During bread making, the content of total phenolic acids and the ferulic acid dehydrodimers was significantly lower in imitated sour dough, dough after mixing, dough after proofing and bread crumbs [86]. Steaming and flaking of dehulled oat groats yielded moderate losses of caffeic acid, while ferulic and vanillic acid increased. Drum drying of steamed rolled oat caused a large decrease in total cinnamic acids [87].

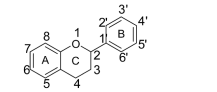
Sinapine, a main phenolic compound of white mustard seed, changed during the traditional stir-baked processing, especially after 15 min, whereby *p*-hydroxybenzoic acid was formed [96]. The content of vanillic acid in Colombian Arabica coffee beans increased in medium- and dark-roasted samples [97]. Storage at 40°C of roselle (*H. Sabdariffa* L.) extract decreased the phenolic compound only by a few percent. After drying and storage at 20°C for 15 wk, 90% of total phenolic compounds remained [102]. In yam Florido (*Discorea alata*), ferulic acid disappeared more slowly when blanching was performed at 60°C than at 65, 70 or 75°C [101]. Artichokes (*Cynara scolymus* L.) were packed in six different films and stored for 8 days at 5°C. After storage, the internal head portion contained more phenolic acid compared to harvest [100].

2.5 Summary: Phenolic acids (hydroxycinnamic and hydroxybenzoic acids)

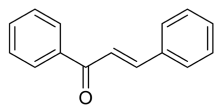
The relevant sources of hydroxycinnamic and hydroxybenzoic acids (phenolic acids) comprise fruits (apples and apple juice, cherries, berries), vegetables (broccoli, lettuce, tomatoes), legumes seeds, cereal grains and their products, wine and coffee beans. The storage process modifies the content of phenolic acids in the plant material; a loss of these compounds as well as an increase in their content was observed. Storage temperature is the major factor contributing to these changes in phenolic acid content. Soaking decreased the content of phenolic acids in legume seeds. The germinated leguminous seeds exhibited changes in content of some phenolic acids. During bread making, oat steaming and flaking, losses of phenolic acids were observed. Freeze-drying did not affect the content of phenolic acids in strawberries. Thermal and SO_2 treatment of blueberries did not result in a change in the content of phenolic acids in pressed juice.

3 Chalcones

The structure of chalcones is given in Fig. 2.



Basic structure and numbering system of flavonoids



Chalcones
Xanthohumol:
4,2',4' = OH; 6' = OCH_3; 3' = Prenyl

Figure 2. Structure of chalcones and basic structure and numbering system of flavonoids. Figure has kindly been provided by Rainer Cermak [1].

3.1 Biosynthesis

Chalcones are key compounds in the biosynthetic pathway of flavonoids. Naringenin is formed by a cyclization of a tetrahydroxychalcon (= chalconnaringenin) which contains a $C_6-C_3-C_6$ skeleton. This cyclization tendency which could be promoted as a result of an improper sample preparation is the reason why this compound could not be documented earlier. Nevertheless, the cyclization of xanthohumol (only existing in hop) yielding isoxanthohumol during beer brewing has a physiological importance.

3.2 Food sources

The following foods are sources of chalcones:

Apples and apple juice [13, 16, 39, 107 – 114], apple cider [112, 115], tomatoes [74, 116], rooibos tea [6, 117, 118], and orange juice [119].

There is no proof of chalcones in orange juice itself, its formation in the gastrointestinal tract will be described in detail.

3.3 Aspects of postharvest processing and storage procedures

The following list gives an enumeration of all the measures or procedures investigated:

Maturity, ripening and time of harvest [74, 115, 116], storage and ageing under different conditions [46, 107, 109, 113], fermentative tea production [6, 117], juice production [13, 39, 111 – 113], differentiation in fractions [13, 107 – 109, 114, 115, 118], peeling [13, 107 – 109, 114], special pressing or extraction [39, 112], freezing [47, 110], enzymatic treatment and maceration [39, 111], and effects of high temperature and oxygen [113].

The observed effects of some of these will be discussed with reference to their application in given foodstuffs in the following sections.

3.4 Structures of individual compounds, content and changes in content in foods

There are known only few structural different compounds of this class of substances in foods. Chalcones, dihydrochalcones and also glycosylated and prenylated structures of the aglycones are specific to this type of compound. The quantitative values are based on HPLC analysis (if no other methods are mentioned).

Glucosylated dihydrochalcones aspalathin and nothofagin are found in particular in rooibos tea but do not occur in all populations [117]. In unfermented plant material, the content ranges from 4 to 12% for aspalathin to 1% for nothofagin. In fermented products however, the contents are much lower (to 0.1 – 0.2%) [6]. Reports in [118] indicated that, depending on the extraction conditions, aspalathin concentrations of up to 13 mg/L in the drink had been observed.

The aglycones, found in apples and apple juice, are the dihydrochalcones phloretin and hydroxyphloretin [108, 115] and the corresponding glucosides, arabinosides [108] and xyloglucosides. Free aglycones were not detected. As usual, content is strongly dependent on variety and the yearly agronomical conditions (*e.g.* weather) prior to harvest. The distribution in the fruit follows the same pattern: the highest concentration was found in the peel, lower concentrations in the flesh and least of all in the apple juice. For example, in the dessert apple Red Delicious [13] amounts of up to 250 mg/kg were determined in the peel, 30 mg/kg in the flesh and up to 20 mg/L in the juice. However, higher concentrations were found in cider apples [112, 115] than in dessert apples. Due to the fact that some compounds are sometimes fixed to flesh components, cloudy juice contains more dihydrochalcon glycosides than clear apple juice [112]. Freshly home pressed juice showed a higher content than commercial varieties [112]. Storage of up to 4 months at 4 or 20°C led to no considerable changes in the content of glycosides while in other phenolic compounds drastic changes were observable. The same is true in the case of enzymatic mash treatment of cloudy apple juice. Nonetheless, the use of high temperatures (80°C) and a high oxygen supply does result in a significant reduction [113].

In tomatoes, only chalconnaringenin was detected and two references were found, [74, 116] for cherry tomatoes from greenhouse production. Seasonal variations are between 100 and 300 mg/kg in fruits harvested with a ripening orange-yellow colour. Postharvest ripening at increasing temperatures (4, 12 and 20°C, at a time for 1 – 3 wk) led to a strong reduction in content.

3.5 Summary: Chalcones

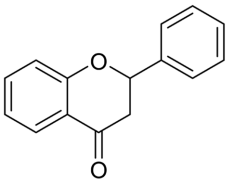
Very few applications deal with chalcones or structurally related compounds. The relevant sources of chalcones are apples, apple juices, tomatoes and roiboos tea. Dihydrochalcones are found in apples and chalconnaringenin in tomatoes. The great tendency of cyclization of the tetrahydroxychalcon (= chalconnaringenin) partly leads to the flavanone aglycon naringenin in common products of tomatoes. Dihydrochalcones in apple and apple juices are more stable. Cloudy juice contains more dihydrochalcon glycosides than clear apple juice and freshly home-pressed juice more than commercially produced juice. Storage at moderate temperatures produced no considerable changes in the content of glycosides but the use of high temperatures led to a clear reduction. The most important prenylated chalcon is currently xanthohumol which occurs in very small amounts only in hops. Maybe the cyclization to isoxanthohumol during beer brewing has a nutritive physiological importance which is mentioned in some papers but not really proofed.

4 Flavanones

The structure of flavanones is given in Fig. 3.

4.1 Biosynthesis

Chalcone synthase catalyses the stepwise condensation of three acetate residues from malonyl CoA with *p*-coumaryl CoA. The *p*-coumaryl CoA is supplied from the phenylpropanoid pathway. The formed naringenin chalcone is then converted into a flavanone form by an intramolecular reaction in which the C-ring is closed by the enzyme chalcone isomerase. Flavanone is probably modified in a stepwise manner to the various derivatives by hydroxylation,



Flavanones
Naringenin: 5,7,4' = OH
Hesperetin: 5,7,3' = OH; 4' = OCH_3

Figure 3. Structure of flavanones. For basic structure and numbering system of flavonoids see Fig. 2. Figure has kindly been provided by Rainer Cermak [1].

methylation, glucosylation and the rhamnosylation. The enzymatically catalysed reaction of the S-isomer is often followed by a chemical reaction which leads to a racemate in ripe fruits.

4.2 Food sources

The following list presents the food sources of flavanones and the papers in which they were discussed:

Oranges [5, 9, 66, 119 – 122], grapefruits [21, 123 – 127], mandarins [125], *Citrus bergamia* juice [128], tangelos [125], lemons [129, 130], pomelos [124], soybeans [99], and tomatoes [72].

4.3 Aspects of postharvest processing and storage procedures

The following list gives an enumeration of all the measures or procedures investigated:

Fruit maturation [130], harvest date [126], storage [5, 126, 127], cold storage [125], squeezing [5, 9], pasteurization [5, 9, 72, 120, 122], extraction [129], concentration [5], freezing [9, 122], freeze-drying [127], light treatment [99], debittering [123], and irradiation [126, 127].

The observed effects of some of these will be discussed with reference to their application in given foodstuffs in the following sections. Maturity of the fruits as well as treatment and storage conditions of the flavanone-containing foods may strongly influence the concentration of individual and total flavanones and also their absorption and antioxidant properties.

4.4 Structures of individual compounds, content and changes in content in foods

The major flavanones discussed in the relevant publications are the aglycones (hesperetin [9, 122, 124] and naringenin [9, 72, 99, 122, 124]), the rutinose glycosides (hesperidin [5, 9, 99, 119, 123 – 125, 129, 130], narirutin [5, 119, 123 – 127], eriocitrin [129, 130] and didymin [5, 125]) and the neohesperidose glycosides (neohesperidin [124, 125], naringin [9, 99, 124 – 127] and poncirin [125, 127]).

The alterations in flavanone glycosides during cold storage (up to 12 or 15 days at 4°C) were determined in segments and juice made from grapefruits, mandarin-type fruits, tangelos and oranges [125]. A significant increase in total flavanones was observed in the fruit segments with storage period. The dominant flavonoid was hesperidin, followed by narirutin and didymin. In contrast, a diminution in total and individual flavanones was found in juices. Three neohesperidose glycosides, mainly naringin, were additionally present in the grapefruit juice. Their concentrations remained unchanged during the storage period. Antioxidant activity correlated with the ascorbic acid content rather than with flavanone glycoside concentration.

The levels of eriocitrin and hesperidin were analysed in leaves, stems and flowers as well as in flavedo, albedo and the pulp of lemons (*Citrus limon*) in dependence on fruit maturation [130]. The highest concentrations were found in albedo. The levels of hesperidin (major flavanone) increased from fruit set until the formation of immature fruits (30 days after anthesis) to 29 – 40% of the fruit dry weight. However, as the fruit grows to maturity, hesperidin levels decrease dramatically to 0.6 – 0.8% of the dry weight. On the other hand, the concentrations of eriocitrin increased with fruit maturity. The maximum level of this flavanone was only 0.6 – 0.8% of the dry weight in mature fruits. In another study, the content of flavonoids (mainly flavanones) and the antioxidative potential was determined in two varieties of lemon juice obtained by either squeezing or by means of two industrial systems [129]. The amount of flavonoids in both types of manufactured juice was at least double that of the hand-squeezed juice. Thus, the predominant flavonoids in juice of the variety 'Fina' were hesperidin (240 – 253 mg/L) and eriocitrin (approx. 200 mg/L) using industrial technology, whereas 104 mg/L hesperidin and 81 mg/L eriocitrin were found after squeezing.

The effects of harvest date, storage and low-dose irradiation on flavanones were investigated in grapefruits [126]. Fruits were treated with 0, 70, 200, 400 or 700 Gy and then stored under simulated storage conditions by subjecting the fruits to 10°C for 4 wk followed by 1 wk at 20°C with 90 – 95% relative humidity. Irradiation and low-temperature storage affected the flavanone content of grapefruit. In general, the early season grapefruit exposed to low doses of irradiation (70 and 200 Gy) followed by storage had significantly higher naringin, narirutin and total flavanone concentrations. An increase in irradiation dose (400 and 700 Gy) resulted in a decrease in flavanones immediately after irradiation. Furthermore, irradiation had different effects on early and late harvested fruits. Total flavanone content in late season nonirradiated fruit was significantly higher than in irradiated fruit after 35 days of storage. Irradiation had no significant effect on the naringin content of late season grapefruit. In general, flavanone concentrations

increased with increasing irradiation dose even in the late season grapefruit, and storage had a positive effect on flavanone levels. Furthermore, the effect of irradiation (300 Gy), storage and freeze-drying on flavanones was determined in grapefruit juice [127]. Interaction between irradiation and storage was observed for the naringin content. In nonirradiated control fruits, the naringin content was 42% higher on day 4 after harvest and decreased then again to the levels of harvest (day 0). After freeze-drying, no changes were found. Storage (6 days) of irradiated grapefruits induced *de novo* synthesis of naringin. This effect was also observed for narirutin and poncerin. In another study, clarified, debittered grapefruit juice was produced by membrane filtration, debittering using an XAD-16 adsorption column and an evaporation process [123]. During this process, more than 78% of the bitterness was removed. Concentration of naringin was reduced from 576 to 2.7 mg/kg. Also some nonbitter flavanones (narirutin, hesperidin) were nearly completely removed.

The main bioactive compounds (flavonoids, carotenoids and vitamin C) were determined in commercial orange juice and in freshly squeezed orange juice [9]. The commercial juices were produced by traditional pasteurization, by short-time pasteurization (for storage at 0–6°C) and by freezing without pasteurization. The highest total flavanone levels were found after traditional pasteurization (123–137 mg/L). The juice prepared by freezing had the lowest total flavanone concentration (36 mg/L). Concentrations of naringenin were between 20 and 37 mg/L (with the exception of frozen juice with 5 mg/L). Hesperidin was highest after traditional pasteurization (approx. 100 mg/L) and lowest after freezing (31 mg/L). Total vitamin C was found to be the major contributor to the antioxidant potential of the orange juices studied, followed by the flavonoids and carotenoids. The influence of high pressure, pulsed electric fields and traditional thermal processing (low pasteurization, high pasteurization, high pasteurization plus freezing and freezing) on bioactive compounds and antioxidant activity was investigated in orange juice [122]. High pressure treatment led to a 20% increase of the naringenin content and a 40% increase of the hesperetin content. Pulsed electric fields had no influence on flavanone content. On the other hand, pasteurization and freezing processes led to a diminished naringenin concentration (16.0%) whereas the hesperetin content was not modified. Total flavanone content was highest in orange juices after the high-pressure treatment (187 mg/L) and lowest after freezing (125 mg/L). High pressure and pulsed electric field technologies were most effective in preserving bioactive substances.

The phenolic compounds were evaluated in orange juices manufactured by squeezing, mild pasteurization, standard pasteurization, concentration and freezing as well as by domestic squeezing [5]. The whole orange juice was divided into insoluble and soluble fractions after centrifugation. Commercially squeezed juice provided 22% more flavanones than home squeezed juice, where the cloud fraction was strongly increased. Particularly, the higher increases belong to hesperidin and didymin, with 30 and 27% increases, respectively, compared to the flavanones extracted by domestic squeezing. The hesperidin content was four-fold higher in commercial samples. Mild and standard pasteurization did not influence the flavanone content in both fractions. After the concentration process of orange juice, didymin decreased by 52% and the other flavanones decreased slightly in the soluble fraction. The cloud fraction was two-fold higher in narirutin, hesperidin and didymin after concentration. The freezing process caused a dramatic decrease in all flavanones in the soluble fraction (23–43%). Freezing and one month storage favours the precipitation of the flavanones to the cloud fraction. Juice pasteurization techniques did not modify the nutritional and antioxidant content of orange juice. However, pulp pasteurization is a restrictive technique that could influence the final content of phenolic compounds. Orange juices made from concentrated and frozen juices provided lower phenolic content than the initial juices. After *in vitro* gastrointestinal digestion of these orange juices, the flavanones able to permeate through a dialysis membrane, and those remaining in the retentate as well as those present in the insoluble fraction were determined [119]. In all juices, a high content of precipitated chalcones (approx. 70% of the total flavanones) was formed under the prevailing physiological conditions. Hand-squeezing led to a higher concentration of flavanones in the permeated fraction and a lower transformation to chalcones than industrial squeezing. Although hesperidin was the most abundant flavanone in all fractions, narirutin and didymin permeated at higher rates. Pasteurization did not influence the solubility and permeability of the flavanones and chalcones, whereas industrial concentration decreased the chalcone formation. Juices made from frozen orange juice contained smaller amounts of soluble flavanones and insoluble chalcones.

The levels of total and individual flavanones (narirutin, naringin, hesperidin, neohesperidin, naringenin, hesperetin) were determined in grapefruits, oranges, pomelos and tangerines commonly consumed in Hawaii [124]. Concentrations of citrus flavanones ranged from 172 to 905 mg/kg. Naringin predominated in grapefruits while hesperidin was highest in oranges. Whether or not flavanone glycosides are less susceptible to degradation during storage was discussed. Storage and processing, especially when heat was applied, generally led to significant flavonoid losses.

The effects of three different methods of processing fresh tomatoes into tomato sauce were analysed with respect to the main flavonoids as well as to antioxidant properties [72]. The methods were: hot break processing at 90°C, cold break processing at 65°C and super cold break processing at 65°C under vacuum. Besides hydroxycinnamates, chlorogenic acid and rutin, naringenin was determined.

Naringenin was strongly affected by processing. Its concentration dropped by about 90% using all three methods.

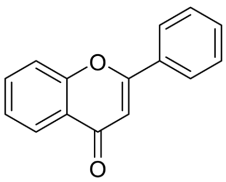
Finally, phenolic compounds were determined in soybean seeds and sprouts grown under both dark and light conditions [99]. Besides other phenolic compounds, naringin, hesperidin and naringenin were found in the seven cultivars used. Despite high variability, more flavanones appeared in sprouts of most green cultivars grown under light conditions.

4.5 Summary: Flavanones

The most relevant sources of flavanones are citrus fruits (especially oranges and grapefruits) and their products. Hesperidin and naringenin are typical flavanones in citrus fruits. The maturity of the fruits as well as their treatment and storage conditions may strongly affect in different manner the concentration of individual and total flavanones and also their antioxidant properties and absorption as shown by several studies within the evaluated period. Thus, the flavanone content in orange juice is influenced by preparation method (*e.g.* squeezing, pasteurization, concentration and freezing).

5 Flavones

The structure of flavones is given in Fig. 4.



Flavones
Baicalein: 5,6,7 = OH

Figure 4. Structure of flavones. For basic structure and numbering system of flavonoids see Fig. 2. Figure has kindly been provided by Rainer Cermak [1].

5.1 Biosynthesis

The basic chemical structure of flavones consists of two benzene rings linked through a heterocyclic pyrone ring. Their biosynthesis starts from cinnamic acid which, with the involvement of monooxygenase and cinnamate 4-hydrolase, is converted to *p*-coumaric acid. The latter, along with 4-coumaroyl/CoA ligase, is transformed to 4-coumaroyl coenzyme A. Condensation of one 4-coumaroyl coenzyme A molecule and three molecules of malonyl-CoA with chalcone synthase as catalyst yields the tetrahydrochalcone. The latter is transformed by Chalcone isomerase into (2S)-flavanone. In most cases, flavones are biosynthesized from this compound with a membrane-bound cytochrome monooxygenase and flavone synthase II as catalyst.

5.2 Food sources

The following list presents the food sources of flavones and the papers in which they were discussed:

Grapefruits [124, 126], buckwheat [131], rooibos tea [117], artichokes [132], honey [133], parsley [134], olives [85, 134, 135], lemons [129, 130, 136], lettuce [137], pepper [137], chicory [137], wine [138, 139], grape juice [7], grapes [124], wheat [140], plums [124], peas [124], bamboo leaves [64, 141], oranges [5, 66, 124, 142], ponkan (mandarin-type) [143], tangerine essential oils [144], broccoli [145], hop [18], cabbage [124], and blueberries [124].

5.3 Aspects of postharvest processing and storage procedures

The following list gives an enumeration of all the measures or procedures investigated:

Grafting [136], time of harvest [130, 137], storage [146], maturity [130], wine ageing [147], geographical origin [144, 147], authentication [133], juice and nectar production [129], distribution in fruit [142, 143], thermal treatment [5, 148], cold-pressing of essential oils [144], extraction procedures [85, 142], and recovery from waste [85].

The observed effects of some of these will be discussed with reference to their application in given foodstuffs in the following sections.

5.4 Structures of individual compounds, content and changes in content in foods

More than 100 flavones have been identified in plants so far. Grafting of the tree rootstock is applied to improve the quality of fruit. The lemon juice obtained from grafted lemon-tree fruits had similar flavonoid content but the flavonoid composition had changed. Depending on the type of rootstock used the content of flavanone 6,8-di-C-glucosyl diosmetin was the most affected [136].

Since flavonoids are secondary metabolites in plants their quantitative and qualitative composition depends on the development stage of a plant, which means that by selecting the harvest time it is possible to obtain products with different flavonoid profiles. It was shown that when the *C. limon* fruits were harvested at the stage II of growth (immature fruit, 30 days after anthesis) they were an excellent source of flavanone hesperidin, but when picked at the stage III (mature fruit 150 days after anthesis) their flavone diosmin [130] content was greatest.

Harvesting time is also a source of flavonoid content variability. In Brazil, the content of flavones in leafy vegetables, like luteolin in lettuce and apigenin in chicory, was higher when vegetables were harvested in the second

semester of the year 2001 rather than in the first semester of the year 2002 [137].

Flavonoid composition of food products may also serve as a tool for food authentication. Profiles of flavonoids determined in Italian wines were shown to be related to the ageing of vineyards rather than to the geographical origin [147]. Similar analysis was suitable for authentication of unifloral Australian eucalyptus honey. The flavonoid profile was genus-specific and comprised tricetin, quercetin and luteolin and quercetin 3-methyl ether, which was proposed as a floral marker for this honey [133].

For obtaining lemon juices three processing methods are used. Simple squeezing of fruits and filtration, 'in line' extraction by squeezing of fruits incised in polar areas with subsequent separation of obtained pulp and juice or two stage extraction – first by means of two rollers turning in opposite directions and second by pressing in screw press and final mixing of the obtained juices. As a consequence, each processing method may have its own characteristics in terms of composition and juice quality [129].

The total flavonoid content of the juices obtained by manual extraction was less than half that obtained by mechanical extraction the percentage of flavones in the juices obtained manually was always lower than in the juices extracted using industrial methods which implies a possible greater contribution of flavones from albedo and flavedo. Indeed, in the mandarin type citrus fruit, ponkan (*Citrus reticulata*), polymethoxylated flavones like nobiletin, tangeretin and sinensetin were found, in decreasing order, in flavedo, albedo and segment membrane, while in juice sacs these compounds were not detected [143]. This is in agreement with the characteristics of flavonoids in the peel of citrus fruits which is rich in polymethoxylated-, C-glycosylated- and *O*-glycosylated flavones [142, 143].

The main processing steps in the production chain include two pasteurization techniques (mild and standard), juice concentration and/or freezing [5]. Pasteurization techniques did not influence the total phenolic content of orange juices. Similarly, no effect was noted in these compounds during juice concentration. In contrast, freezing resulted in dramatic loss in juice phenolics (–35%) which could be due to storage and thawing. In the monitored orange juices two flavones were found, vicenin 2 (apigenin 6,8-di-C-glucoside) and one luteolin derivative. There was a loss in the content of boths flavones during freezing (around 20%) while no changes were observed during pasteurization and concentrating. The mandarin type Dancy tangerine (*Citrus tangerine* Hort. Ex Tan.) cultivated in Mexico is the raw material for essential oil [144]. Analysis of industrially produced cold-pressed oils from Dancy tangerine from different regions of Mexico revealed the presence of five polymethoxylated flavonoids: tangeretin, hepta-methoxyflavone, nobiletin, tetra-*O*-methyl-scutellarein and sinensetin, with the first dominating. It is worth noting that the sample from the mountain region had the lowest polymethoxylated flavone content while the oil from the fruits from the tropical region had the highest.

By-products of fruit and vegetable processing are also an interesting source of bioactive compounds. The peel of Greek Navel oranges (*Citrus sinensis*) is considered to be a food additive of natural origin or a pharmaceutical supplement with antioxidative properties [142] because of its polymethoxylated flavones, C-glycosylated flavones, *O*-glycosylated flavones and flavanones. With regard to the C-glycosylated flavones three compounds were identified, 6-C-β-glucosyldiosmin, 6,8-di-C-glucopyranosylapigenin and 6,8-di-C-β-glucosyldiosmin.

Olive processing also generates waste which can be utilized as a source of bioactive phytochemicals for food and pharmaceutical applications [85]. Different solvent combinations were tested and extraction at ambient temperature was found to provide a compromise between recovery of phenols and their degradation. Identification of the obtained extract revealed the presence of two compounds belonging to the group of flavonoids, namely flavonol glycoside rutin and flavone luteolin.

5.5 Summary: Flavones

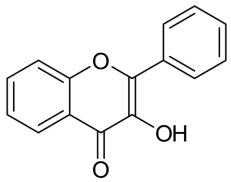
Flavones are one of the principal classes of flavonoids and more than 100 flavones have been identified in plants so far. Citrus fruits, parsley, lettuce, chicory and grapes are the richest source of flavones in the human diet. Since flavonoids are secondary metabolites in plants, their quantitative and qualitative composition depends on the developing stage of a plant, which means that by selecting the harvest time it is possible to obtain products with different flavonoid profiles. Also other agronomical treatments (such as grafting) are a source of flavone content variability. The total flavonoid content in citrus juices obtained by squeezing was less than half that obtained by industrial methods, the percentage of flavones in the juices obtained manually was always lower than in the juices extracted by industrial methods which implies a possible greater contribution of flavones from albedo and flavedo. Recognition of flavone distribution within a plant/fruit permits a range of products with different flavone characteristics to be obtained from a single source.

6 Flavonols

The structure of flavonols is given in Fig. 5.

6.1 Biosynthesis

Hydroxylation of flavanones on C3 generates hydroxyflavonols, the direct precursors of flavonols.



Flavonols
Kaempferol: 5,7,4' = OH
Quercetin: 5,7,3',4' = OH

Figure 5. Structure of flavonols. For basic structure and numbering system of flavonoids see Fig. 2. Figure has kindly been provided by Rainer Cermak [1].

6.2 Food source

The following list presents the food sources of flavonols and the papers in which they were discussed:

Plums [12, 124, 149–152], apples [13, 41, 107–110, 112, 114, 115, 124], onions [124, 137, 153–161], blueberries [14, 29, 58, 96, 124], cranberries [162, 163], currants [164, 165], blackberries [13], raspberries [4], chicory [137, 166], grapes [2, 13, 124, 167–169], wine [2, 49, 51, 53, 54, 139, 147, 170–173], tomatoes [72, 74, 116, 124, 157, 174], strawberries [8, 13, 63, 64, 124, 158, 175–177], buckwheat [131, 178], sorghum [88], potatoes [76, 124], mangos [124, 179, 180], kernels [138], cherries [13, 42, 43, 181], spinach [124, 156, 182], kale [183], broccoli [124, 145], cabbage [55], asparagus [156], lettuces [124], oranges [124], arugulas [137], tea [184], artichokes [132], coffee [185], honey [133, 186], apricots [187], citrus fruits [136], oranges [142], bush butter fruits [188], radishes [156, 189], cabbages [156, 124], pepper [156], olive oil [85, 190], cowpeas [104], cactus pears [191], *Kancolla* seeds [192], Pinto beans [193], beans [82], soybeans [99], and lentils [82].

6.3 Aspects of postharvest processing and storage procedures

The following list gives an enumeration of all the measures or procedures investigated:

Maturity, ripening and time of harvest [63, 74, 82, 99, 115, 116, 176, 178, 179, 188, 189, 194], storage and ageing under different conditions [2, 4, 14, 41–43, 49, 53, 76, 149, 152, 153, 169, 170, 173, 181, 185, 189, 193, 195], fermentation [2, 51, 55, 61, 104, 139, 168, 172, 173, 184], juice production [2, 58, 112, 142, 162, 181, 191, 196], differentiation in fractions [114, 167, 174], peeling [109, 142, 153, 180], special pressing or extraction [2, 64, 72, 165], enzymatic treatment and maceration [51], irradiation [53, 158, 175, 183], drying [8, 102, 132, 152, 159], sensory effects [51], germination [88], browning-blanching and polyphenoloxidase (PPO) activity [124], proveniences [8, 12, 63, 107, 110, 112, 133, 138, 145, 147, 150, 151, 156, 164, 180, 186, 187, 194], and cooking [76, 160, 166, 182, 190].

The observed effects of some of these will be discussed with reference to their application in given foodstuffs in the following sections.

6.4 Structures of individual compounds, content and changes in content in foods

The flavonol distribution, typical for each species (as for total flavonoids), is due to intrinsic and extrinsic factors. Some are related to the existence of synthesis and regulation pathways controlled by enzymes, others are connected to habitat (such as season and climate conditions) and degradation caused by human activities (such as degree of ripeness, cultural practices, food preparation and processing).

Peeling, skinning, trimming, depitting and/or leaf selection may cause a partial or total decrease in flavonol levels: data obtained, analysing the flavonol content in the different layers of onion bulbs (skins, outer fleshy layer, edible portion), show that, after normal household peeling, 79% of the total content of quercetin 4′-glucoside is still present in the edible portion [153].

Quercetin-3-rutinoside, kaempferol-3-rhamnosyl-hexoside and quercetin-3-acetyl-hexoside were found only in the peel tissue of apricots from different cultivars [187]. Small amounts of kaempferol (27 ± 2 μg/kg edible fruit pulp) occur only in the pulp of the cactus pear [191].

The flavonols, found in fruits and vegetables, are present in glycosylated form, mainly as *O*-β-glycoside with a sugar moiety at the C-3 position. The number and type of sugar residues is diverse. Flavonol glycosylation does increase the solubility promoting their accumulation in plant cell vacuoles.

Quercetin-3-arabinoside was found in both furanose and pyranose forms in cranberries [163]. The quantification and the screening of mango flavonol glycosides could be used as a control parameter for mango puree concentrate production [180].

The exposition to light stimulates the flavonol biosynthesis in a few plant organs causing them to collect in the outer and aerial tissues (skin and leaves). A strong divergence between the flavonol values obtained for apple peel and pulp was noticed: quercitrin (94.0 ± 36.0 *vs*. 7.76 ± 1.95 mg/kg FW), reynoutrin (48.9 ± 16.2 *vs*. 1.98 ± 0.50 mg/kg FW), avicularin (110 ± 32.9 *vs*. 2.27 ± 0.46 mg/kg FW) [109].

Domestic cooking methods such as boiling, microwaving, frying and steam-cooking could cause a loss of the flavonols in potatoes [76]. Onions (*Allium cepa* L.) have been prepared by sautéing, baking and boiling: after baking and sautéing, quercetin concentrations increased by 7–25%, while boiling led to a decrease of 18% [160]. Quercetin derivatives such as quercetin-3,4′-glucoside and quercetin-4′-glucoside are not deglucosylated during cooking. In addition, more than 50% of flavonoids and other soluble plant materials are readily transferred from onions into soup during cooking [155].

A loss in flavonol content was observed in several vegetables and fruits during boiling and blanching [124]. Quercetin and kaempferol glycoside levels do not change in fresh or frozen, store-bought or home-grown red raspberries [4].

A lot of investigations were conducted on the influence of processing procedures and techniques on food, especially juice processing and vinification. Very low concentrations (0.4 – 4 mg/L) of quercetin derivatives in dessert apple juice have been found, while in cider apple juice quercetin glycosides reached concentrations of up to 27 mg/L. No free quercetin was found in the apple juice [112]. In blueberry juice produced by three different types of treatment (initial heat treatment, SO_2 and no treatment), polyphenolic levels remain similar [58].

Myricetin, quercetin, kaempferol and isorhamnetin as 3-*O*-glycosides were found in *Vitis vinifera* grapes. In addition, the presence of their aglycone forms in wine shows that processes such as vinification, maturation and/or aging of wine stimulate the hydrolysis of the glycosides [171]. The higher flavonol content in enzyme-treated wines seemed to be connected to the higher degradation of pulp caused by the enzymes [51]. In [197], it have carried out an investigation on the production of juice and wine through hot- and cold-pressed techniques, and on the production of wine following on-hull fermentation for 3, 5 and 7 days. The results obtained from the comparison between hot-pressed juice and on-hull fermentation wine showed a decrease in myricetin, quercetin and kaempferol levels during storage at a rate consistent with temperature. The loss was 45 – 63% at 20°C and 75 – 79% at 37°C, depending on processing method.

Reports in [72] have described the influence of three different processing methods, hot break, cold break and super cold break on the flavonol concentration in an Italian tomato variety: rutin concentration, the main flavonol identified, after the cold break process, increased from 154.47 ± 19.8 to 239.81 ± 19.8 mg/kg dry weight.

Kaempferol has been identified as the principal flavonol in the dried aerial parts of tea (*Sideritis euboea*) [183]. The major flavonol that occurred in Pu-er tea (a treated fermented tea produced from crude green tea prepared from the leaves of *Camellia sinensis*) is myricetin [184].

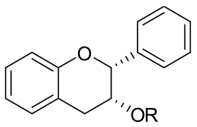
The influence of storage on the flavonol content of foods is ambiguous. Analysis of cherry juice from five different cultivars revealed the presence of quercetin glycosides at concentrations of 31 – 109 mg/L. No variations were found after 180 days of storage at 20°C [181]. Storage at 22°C for 36 wk increased the total phenolic concentration in red and yellow onions [154]. Cold storage for up to 3 days led to relatively small changes in the concentration of the different antioxidants in strawberries [63].

6.5 Summary: Flavonols

The relevant sources of flavonols for human nutrition are plums, apples, onions and blueberries. The main representative molecules are quercetin and kaempferol. Peeling, skinning, trimming, depitting and/or leaf selection may cause a partial or total decrease in flavonol levels. Changes were found also in flavonol levels with different cooking methods. The influence of food storage on the flavonol content of foods is ambiguous: increases, decreases and no change have been observed depending on the storage conditions, phytochemical stability characteristics and quality of the food analysed. A lot of investigations have focused on the influence of processing procedures and techniques on food, especially juice processing and vinification. Vinification, maturation and/or aging of wine were found to stimulate the hydrolysis of the glycosides.

7 Monomeric flavanols (catechins)

The structure of monomeric flavanols is given in Fig. 6.



Flavanols
Epicatechin: 5,7,3',4' = OH; R = H
Epigallocatechin: 5,7,3',4',5' = OH; R = H
Epicatechin-3-gallate: 5,7,3',4' = OH; R = gallate
Epigallocatechin-3-gallate: 5,7,3',4',5' = OH; R = gallate

Figure 6. Structure of monomeric flavanols. For basic structure and numbering system of flavonoids see Fig. 2. Figure has kindly been provided by Rainer Cermak [1].

7.1 Biosynthesis

During the biosynthesis of the monomeric flavanols (catechins) there is a strong relation between the monomer catechins and the resultant proanthocyanidin oligomers. They nearly always occur together in plants. Proanthocyanidin oligomers do have special properties. Consequently there is another paper dealing with them within this issue [198].

Normally, stereo specific (+)-catechin and (–)-epicatechin are synthesized in nature. However, newer chiral analysis show that in some cases, their enantiomers could have also been either naturally formed (*e.g.* in guarana) or formed during processing (*e.g.* in chocolate).

7.2 Food sources

The following list presents the food sources of catechins and the papers in which they were discussed:

Grapes, grape juice and grape seeds [7, 15, 17, 52, 121, 167, 197, 199, 200–207], wine and winery by-products [15, 17, 51, 52, 119, 201, 203, 208–211], tea leaves and tea [204, 207, 212–215], Pu-er tea [184, 216], apples [11, 41, 137, 197, 217], cocoa [21, 218], coffee [185], cherries and cherry juice [42, 43, 181, 197], acerola juice [219], pears [45], strawberries [175], yams and potatoes [101, 220], common beans [221], lentils [222], wheat fractions [19], corn, chips and tortillas [223], hawthorn leaves [224], *Crataegus pinnatifida* fruits [225], pale malt [226], shea kernels and shea butter [138], fruits, vegetables, beans, nuts and cereals [227], vegetables, spices and dressings [33], and *Argania spinosa* oil and press cake [103].

The following publications are also overviews: [201] (88 foods), [197] (28 fruits) and [137] (Brazilian vegetables and fruits). In [228], an addition of epicatechin to bovine milk is described.

7.3 Aspects of postharvest processing and storage procedures

The following list gives an enumeration of all the measures or procedures investigated:

Maturity, ripening and time of harvest [15, 41, 42, 137, 181, 210, 219], storage and ageing under different conditions [15, 41–43, 52, 181, 185, 209, 211, 229, 230], fermentation [7, 15, 17, 51, 52, 184, 185, 199, 203, 209–213, 216, 230, 231], juice production [7, 181, 219], differentiation in fractions [19, 137, 167, 199, 217, 221, 222], peeling [17, 137, 167, 199, 217], special pressing or extraction [51, 203, 204, 231], enzymatic treatment and maceration [7, 51, 211], irradiation [175, 214], acidification [223], 1-methylcyclopropene (MCP) treatment [41], drying [45, 101, 213, 225], sensory effects [232, 233], brewing [131, 215], cooking and steaming [33], stress treatment [224], browning-blanching and PPO activity [101], and provenience [138].

The observed effects of some of these will be discussed with reference to their application in given foodstuffs in the following sections.

7.4 Structures of individual compounds, content and changes in content in foods

For the determination and judgement of the monomeric flavanol content attention must be paid to its formation from or transformation into proanthocyanidins (see Section 7.1). The quantitative values are based on HPLC analysis (if no other methods are mentioned). Different standards are sometimes used as base for the quantitative analysis of the same substances in different publications. It can be necessary, *e.g.* if single compounds are not available. That means that results can only be partially considered as absolute values.

All related publications contain information on the content of catechin and epicatechin. Furthermore, gallocatechin, epigallocatechin, catechinglucoside [222], epigallocatechinglucoside [184], gallocatechin gallate [138], epigallocatechin gallate [138] and epicatechin gallate [203, 206, 231] are mentioned. In [184], new 8-C substituted flavanols (puerins) are described. New oxidation products of catechin and epicatechin (viniferones) have been identified [205]. The catechin contents given in [19, 197, 210, 217] have been determined by unspecific colour reactions, such as Folin-Ciocalteu assay.

All the relevant publications show that content is strongly dependent on variety and the yearly agronomical conditions prior to harvest. The distribution in the fruit also follows the normal pattern: the highest concentration was found in the peel, lower concentrations in the flesh and even less in the juice.

In grapes and the corresponding wines, the content is influenced by fermentation: in [199], up to 30 mg/L of monomeric catechins in wine, 140 mg/kg in the skins and 1500 mg/kg in the seeds are reported. Grape seeds and winery by-products are recognized as good sources of flavanols. In [203], hot extractions are described, in [231], extractions with supercritical liquids and/or extractions under high pressure and modified temperature. The use of subcritical water extraction [231] partially gave better results than mixtures of methanol and water.

Different cultivars of cherries [42, 43] with greatly varying catechin content were investigated under cold storage (2°C) and room temperature (15°C) conditions. In juice, a decrease at 15°C was observed. Ripe apples treated with MCP [41] and stored at different temperatures and time periods showed no significant variations in catechin concentration. In immature and mature acerola juice [219], a decrease in total polyphenols (calculated as catechin) was found (3800–2400 mg/kg). In strawberries [175], the content of catechin and especially of epicatechin was markedly reduced by irradiation whereas the concentration of flavonols (quercetin derivatives) was not affected.

In two cultivars of yams, the effect of blanching at different temperatures was investigated. Catechin was found to decrease during blanching, independent of temperature. No epicatechin was detected in yams or potatoes [101, 220]. The production of chips and tortillas from white and blue corn resulted in big losses of total polyphenols [223]. In [232], the addition of epicatechin to bovine milk is described. Epicatechin inhibited the thermal development of aroma compounds in ultrahigh-temperature processing of milk.

7.5 Summary: Monomeric flavanols (catechins)

Important sources of catechins for human nutrition include tea, grapes, red wine, cocoa and chocolate. The most common flavanol monomers are catechin and epi(gallo)catechin and their gallates (in tea). The catechins are widespread in the plant kingdom and there is a strong relation-

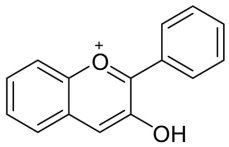
ship between the monomeric catechins and the resultant proanthocyanidin oligomers. They nearly always occur together in plants. Due to the large number of references dealing with the oligomers, the proanthocyanidins will be discussed in a further overview of the COST action 926 [198].

Normally, (+)-catechin and (−)-epicatechin are synthesized in nature. However, more recent chiral analyses show that in some cases, their enantiomers could have also been either naturally formed (*e.g.* in guarana) or formed through technological treatment (*e.g.* in chocolate). It has been supposed that the bioavailability of (−)-catechin was not as good as that of (+)-catechin.

Flavanols are good substrates for the PPO, therefore they are often decreased by this reaction. On the other hand, fermentation of grapes increases the content in their resultant wines. It is known that grape seeds and winery by-products are good sources of flavanols (see also in the section of proanthocyanidins). Different effects of storage (time and temperature) were observed with different fruits and their products. In general high temperature decreases the content. In strawberries, the catechin content, in particular epicatechin, was markedly reduced by irradiation whereas the concentration of quercetin derivatives was not affected.

8 Anthocyanins

The structure of anthocyanins is given in Fig. 7.



Anthocyanidins
Delphinidin: 5,7,3',4',5' = OH
Malvidin: 5,7,4' = OH; 3',5' = OCH_3

Figure 7. Structure of anthocyanins. For basic structure and numbering system of flavonoids see Fig. 2. Figure has kindly been provided by Rainer Cermak [1].

8.1 Biosynthesis

The $C_6-C_3-C_6$ skeleton is formed involving the polyketide and shikimate pathways yielding flavanones as the first products of flavonoid biosynthesis. Subsequently, these are converted to dihydroflavonols which serve as educts for the formation of leucoanthocyanidins (flavan-3,4-diols, intermediate product) and anthocyanins, respectively. Several hundred anthocyanin structures are known which result from substitution of the anthocyanidins with hydroxyl and methoxy functions at various stages of the flavonoid biosynthesis. Finally, the aglycones are glycosylated with saccharide moieties, such as glucose, galactose, xylose, arabinose and rhamnose, but also with di- and triglycosides. In many cases, the saccharide moieties are acylated with aliphatic or aromatic acids, substitution with the latter giving rise to enhanced pigment stability due to intramolecular copigmentation of, *e.g.* red cabbage, black carrot, red radish and red and purple potato anthocyanins [234–236]. In addition, complex reactions of anthocyanins during food processing and storage, *e.g.* during wine aging, lead to a vast number of novel compounds, the structure and content of which, in processed foods, are still largely unknown [207].

8.2 Food sources

The following list presents the food sources of anthocyanins and the papers in which they were discussed:

Grapes and red wine [2, 15, 49, 51, 52, 54, 58, 64, 121, 122, 168, 171–173, 207–209, 211, 230, 237–270], strawberries and strawberry jam [158, 271–278], prunes [152, 279], cherries and cherry juices [23, 42, 43, 181], plums and plum purée [228, 262, 280], pomegranates and pomegranate juice [281–284], mangos [180, 285], red oranges [286–289], cherry laurel [44], mulberries [290, 291], apples and apple juice [39, 41, 108, 113, 114, 196, 217, 292], litchis [293–296], raspberries [4, 275, 297, 298], blackberries [298–301], black chokeberries [302], blueberries and blueberry juice [14, 58–60, 106, 275, 303, 304], black currants [165, 305–309], bilberries [300, 307], cranberries [310, 311], bayberries [65], berries of *Ribes* species [164], *Annona cherimola* fruit [312], black carrots and black carrot juice [235, 313–315], red and purple potatoes [236], corn kernels [223], asparagus [316], roselles [102, 317], olives [318, 319], red onions [153], kidney beans [320], snowball tree fruits [321], sweet potatoes [322], sorghum [88], fruits, vegetables, beans, nuts, cereals and foods [227, 323], and Indian diet [324].

8.3 Aspects of postharvest processing and storage procedures

Anthocyanin stability and changes in the anthocyanin profile have been investigated using a number of different conditions and treatments during storage and postharvest processing:

Winemaking, wine aging and storage [2, 49, 51, 54, 122, 171, 173, 207–209, 211, 230, 238, 241, 243, 246, 248, 252–254, 257–260, 264–270], maturity stage and postharvest ripening [15, 42, 266, 277, 279, 301, 303], freezing and cold storage [4, 23, 43, 113, 237, 247, 251, 274, 279, 281, 289, 295, 297, 299, 303, 325], thermal treatment and storage at increased temperatures [44, 59, 102, 223, 228, 244, 276, 286, 291, 293, 305, 306, 313–316], comminution and pressing techniques [304], clarification, filtration and concentration [58, 65, 283], juice production [324], enzymatic treatment [264, 280, 307], peeling [153], extraction

[21, 121], drying [102, 152, 178, 249, 303, 326], fermentation [254, 276, 304], storage [272, 287], pre- and postharvest dip treatment or spraying [273, 281, 292–295, 311], MCP treatment [23, 41], germination [88], postharvest UV irradiation [158, 250, 284, 308], controlled and modified atmosphere (MA) storage [14, 296, 310, 312, 318, 319], anthocyanin structure [234–236], and copigmentation effects [52, 172, 240, 256, 261, 327].

The observed effects of some of these will be discussed with reference to their application in given foodstuffs in the following sections.

8.4 Structures of individual compounds, content and changes in content in foods

Among the flavonoids, anthocyanins are the most important class of phenolic compounds and have thus been intensively studied with special regard to their techno-functional and biofunctional properties. For this reason, almost every third paper evaluated in this review deals with anthocyanins, either exclusively or in combination with further phenolic compounds.

In order to improve storage behaviour and to maintain quality attributes, fruit and vegetables should be harvested at optimum maturity. Strawberries, which are harvested at their colour break or half-coloured status, will redden during storage, but their pigment content will remain below that of strawberries ripened on the field and harvested at commercial maturity as has been shown for the major strawberry anthocyanins, pelargonidin 3-glucoside and cyanidin 3-glucoside. Thus, colour development during postharvest ripening is significantly affected by the initial pigment content [277]. Even fruits harvested at commercial maturity may exhibit increases in anthocyanin content throughout cold-temperature storage. The amount of pigment in prunes increased 1.5–1.7-fold during storage at 1 and 5°C, respectively [279], and an up to five-fold increase in the pigment content of cherries stored at 15 ± 5°C was observed. Higher temperatures enhanced anthocyanin biosynthesis during storage [42]. The change in the content of individual phenolics of various cherry cultivars during storage also affected *in vitro* antioxidant effects on human low-density lipoproteins [43]. Furthermore, the choice of suitable cultivars is of particular importance for storage. Jams produced from various strawberry cultivars differed in terms of pigment and antioxidant capacity retention. While temperature proved to be the most important factor during storage, significant differences between jams stored under light or in the dark were not observed [272].

The temperature during storage strongly affects the half-life values of anthocyanins in a fermented black carrot juice, which ranged from 231 to 239, 55 to 80 and 21 to 22 days, when the product was stored at 4, 25 and 40°C, respectively [313]. However, the stability of individual anthocyanins is not only dependent on their structure, but also on matrix components which might reduce pigment degradation, such as colourless phenolic compounds. Black carrot extracts exhibited varying stabilities in fruit juices and nectars when stored at temperatures up to 37°C or heated at 70–90°C [314]. Cherry juices stored at 20°C in the dark showed a loss of 50% of their monomeric pigments after 180 days [181]. In contrast, the amounts of cyanidin galactoside in apple juices stored at 4 and 20°C, remained virtually unchanged for a period of up to 30 days [113]. The loss of monomeric anthocyanins during seven months of storage in the dark was up to 88% for conventional red wine and 91% for ecological red wine [49], and even higher degradation rates may be observed in grape skin extracts [237]. The colour loss of a plum extract stored at 25°C for 138 days amounted to 21 and 23% at pH 1 and 3, respectively, while under the same conditions a grape extract showed colour losses of 30 and 31% [262].

There is increasing interest in the stability of individual phenolic compounds to provide processes and optimize conditions for maintaining polyphenol levels in plants or products derived thereof or minimizing loss of phenolics, *e.g.* by cold-temperature or frozen storage. The anthocyanin content of raspberries (mainly cyanidin glycosides and minor amounts of pelargonidin glycosides) was not affected by freezing. Furthermore, 3 days of storage at 4°C and at 18°C for 24 h had no significant effects on the levels of individual anthocyanins. Since anthocyanins were quantitatively predominant and further compounds showed only minor changes, these storage conditions did not affect the antioxidant capacity either [4]. These findings were corroborated for wild blackberries. Antiradical activity showed only a slight decrease throughout storage, and a correlation with anthocyanin and total phenol content could be established [299]. However, long-term storage at −18°C (12 months) and short-term storage with temperature fluctuations between −18 and −12°C (24 days) were shown to significantly affect raspberry surface colour in terms of *L*a*b** values. Interestingly, an increase in the total anthocyanin content was observed under the latter conditions [297]. In contrast, the loss of total anthocyanins in strawberry fruit amounted to 40.2% (−12°C), 34.3% (−18°C) and 17.6% (−24°C), respectively, after 90 days of storage. The method of freezing is obviously important for pigment retention, since strawberries frozen quickly showed lower anthocyanin levels than samples frozen slowly [247].

The shelf-life extension of fresh fruit is usually achieved by low-temperature storage. Cold storage may be combined with postharvest dip treatments of fresh fruit, such as litchis, with chitosan or Carnauba wax to prolong shelf-life [295]. Blueberries could be stored up to 7 wk at 5°C; however, the storage period was cultivar-dependent. Anthocyanin content and antioxidant activity remained unchanged during storage. One cultivar, the berries of which were harvested before commercial maturity, even exhibited an increase in pigment amounts (1600%), total phenolic con-

tent (40%) and antioxidant activity (79%) during the first 3 wk of storage, which was only partially due to water loss [106]. Strawberry fruit stored at 0, 5 and 10°C, respectively, did not show temperature-dependent differences of surface colour, however, *L*a*b** values changed with storage time. In contrast, total anthocyanin content was significantly affected by the temperature and storage period, with fruit stored at 10°C actually exhibiting a gradual increase in pigment, while storage at 0°C caused pigment loss throughout the period of 91 days. Even though pigment retention was higher at 10°C, overall fruit quality was better maintained at 0°C [251]. Pigment loss in strawberries may amount to up to 43% during 8 days of storage at 1°C unless the fruit are wrapped to avoid water loss, which might have contributed to enhanced pigment breakdown along with oxidative mechanisms caused by increased PPO activities in unwrapped strawberries [274]. Pigment loss was also shown to be minimized by postharvest application of methyl jasmonate in combination with an ethanol treatment, because the anthocyanin content of the fruit exhibited a reduced downward trend when compared to the control fruit [273].

When cherries were stored at 2–4°C for 12 days without MCP treatment, no change in anthocyanin content was observed. In contrast, MCP-treated fruit exhibited a 9% decrease (360 μg/kg MCP) and a 12% increase (180 μg/kg MCP), respectively, after 12 days of cold storage [23]. Anthocyanin levels of pomegranate fruit stored at 5°C were shown to significantly increase during the first month of storage, probably due to continued pigment biosynthesis after harvest. Thereafter, a steady downward trend was observed, the extent of which was dependent on postharvest treatment of the fruit (spraying with wax and $CaCl_2$ solutions). Individual compounds (mainly cyanidin and delphinidin glycosides and minor amounts of pelargonidin glycosides) showed almost the same trend throughout storage [281]. Increasing anthocyanin content has also been observed for red oranges stored at 4°C. Pigment content was eight times higher than those of control fruit stored at 25°C. This was traced back to an enhanced expression of the structural genes involved in anthocyanin biosynthesis, such as PAL, chalcone synthase, dihydroflavonol 4-reductase and UDP-glucose flavonoid glucosyl transferase due to the cold stress [289]. In general, low temperature storage has been shown to enhance postharvest phenolic metabolism in a wide variety of plant matrices. There is a special low temperature below which the phenylpropanoid metabolism is stimulated; however this temperature varies from commodity to commodity [325].

Thermal treatment, such as blanching, pasteurization and sterilization, are the most common methods for preserving food, but has significant effects on the content of unstable compounds, such as anthocyanins. Blanching has been shown to increase anthocyanin yields during juice processing by inactivating deteriorating enzymes and increasing fruit skin permeability [59]. Steam blanching and subsequent immersion into high-acid solutions have also been applied for the colour stabilization of litchi pericarp, since litchi anthocyanins are highly susceptible to rapid degradation after harvest [293].

The heating of black currant anthocyanins in aqueous solution revealed high pigment stability at 75°C (insignificant loss after 150 min) and a decrease in colour intensity at higher temperatures (20 and 45% decrease at 85 and 95°C) with cyanidin rutinoside being the most stable black currant pigment. Furthermore, the presence of high amounts of sucrose had positive effects on pigment stability during heating, whereas fructose showed the opposite effect [306]. Rather obsolete traditional processes, such as the production of pekmez from cherry laurel (*Laurocerasus officinalis* Roem.) by boiling the juice in copper containers over an open wooden fire, resulted in high losses of monomeric anthocyanins amounting to up to 92.5% [44]. The heating of mulberry extracts at 90°C gave rise to a rapid decrease in monomeric anthocyanins and a concomitant formation of polymeric pigments. This study also showed that high saccharide content has to be considered when solutions containing anthocyanins are thermally treated because of the formation of intermediates (such as furfural and hydroxymethylfurfural) and end products of the caramelization reaction which might interact with anthocyanins yielding an enhanced pigment loss [291].

Experiments performed with plum purée at 50–90°C showed first order reaction kinetics for anthocyanin degradation. The activation energy for anthocyanin breakdown was found to be 37.48 kJ/mol [232]. First-order kinetics of pigment decomposition was also shown for blood orange. Blood orange anthocyanins are highly susceptible to degradation at elevated temperatures. Half-lives ranged from 6.3 to 1.5 h for juice, 3.4 to 0.7 h for a concentrate of 45°Brix and 2.0 to 0.4 h for a concentrate of 69°Brix, respectively, with solutions of higher total soluble solid content showing higher relative pigment losses [286]. Nixtamalization, a particular form of food preparation common to Mesoamerican cultures, has also been shown to be detrimental to anthocyanins. This process consists of alkaline cooking of corn kernels in a lime or calcium hydroxide solution, which imparts typical organoleptic characteristics to the final products. The lime cooking and subsequent thermal processing to produce tortillas and tortilla chips revealed pigment losses of >50%. Acidification of the nixtamal after alkaline cooking by adding fumaric acid significantly reduced pigment losses, thus confirming the known pigment instability under alkaline conditions [223].

In contrast, preharvest heat treatment may also be applied to effectively inhibit anthocyanin biosynthesis. Pigment formation, *e.g.* in asparagus, spears during storage can be avoided by immersion in hot water (50–55°C) [316].

The drying of fresh plant material has long been used to obtain stable products which can be stored for an extended

period of time by reducing a_w values and thus minimizing chemical and enzymatic reactions as well as microbial spoilage. The drying temperature is of particular importance for pigment retention as could be shown for prunes. Significant differences in the anthocyanin amounts were observed when prunes were dried at varying temperatures, 85, 70 and 60°C. Lower temperatures led to lower pigment degradation [152]. These findings were corroborated by another study comparing the effects of different drying methods, such as microwave-vacuum drying and freeze-drying with convective drying, on strawberry pigment content. Methods which exerted lower thermal impact on the fruit yielded products with higher anthocyanin content [278]. Osmotic pretreatment prior to drying may give rise to higher pigment losses as has been shown for blueberries. Cabinet drying resulted in a 41% decrease in anthocyanin amounts, whereas a combination with the soaking in high-sugar solutions led to a 49% loss, probably as a result of leaching effects during the pretreatment [303]. The drying of roselle petals at elevated temperatures (75°C compared to 50 and 25°C) and storage at 40°C for 15 wk caused a loss of only 15% of total phenolics, whereas the portion of anthocyanins decreased from ~80% to ~50% of the total phenolics indicating higher susceptibility of the pigments to thermal degradation or formation of oligomeric and polymeric products [102]. In contrast, partial drying of grapes for the production of dessert wines may also be responsible for their increased anthocyanin content which cannot simply be attributed to concentration due to water loss. Stimulation of metabolic pathways as a consequence of the stress situation during the slow tunnel-drying procedure must also play a role [249]. Thus, dehydration may be a suitable method for maintaining high anthocyanin levels [95].

Among other functions plant phenolics act as UV filters protecting plant tissues from damaging radiation. Thus, enhanced biosynthesis of polyphenols is a common response to UV irradiation during plant growth. However, UV irradiation of fruit and vegetables as a postharvest treatment has also been shown to improve quality throughout storage, *e.g.* by increasing anthocyanin levels in strawberries [158]. In another study UV-C irradiation doses of 4.1 kJ/m^2, alone or in combination with heat treatment at 45°C, significantly increased anthocyanin amounts in strawberries, but nonirradiated control samples showed an even higher pigment content. This might be ascribed to the differing effects of low and high UV-C doses on the rate of PAL expression [250]. Therefore, further studies are still required to investigate effects of various irradiation doses on strawberries of several cultivars and ripening stages. In contrast to these results, ready-to-eat pomegranate arils did not show a change in their anthocyanin content when irradiated with UV-C doses ranging from 0.56 to 13.62 kJ/m^2 compared to the control samples, whereas the harvest date significantly affected several quality parameters at the end of the storage trials over 15 days under MA [284].

UV irradiation of processed products has a detrimental effect on pigment stability. Irradiating black currant extracts with UV light (40 W) at 40°C led to a 45 – 55% loss of cyanidin and delphinidin glycosides after 4 h [308].

Anthocyanin stability may also be significantly affected by the atmosphere composition. Although the total phenolic content of cranberries was not affected by CA storage, a moderate effect of the CO_2 concentration (but not of the O_2 levels) on the content was observed. However, individual compounds were not assessed [310]. Blueberries stored in air or high-O_2 (40 – 100% O_2) atmospheres did not show significant differences in their lightness throughout storage, whereas at oxygen concentrations ≥60% hue angles were increased after 4 and 5 wk of storage, indicating a more intense blue colour. Similarly, the total anthocyanin content increased 1.2-fold after 35 days of storage under the same conditions, thus exhibiting a significantly higher content and ORAC values compared to 40% O_2- and air-treated fruit. The same observations were made for the nine individual anthocyanins detected in the blueberries [14]. CO_2 atmospheres or anaerobic conditions are also employed for the postharvest treatment of fruit and vegetables. Black ripe table olives showed a rapid loss of monomeric anthocyanins within 15 days of anaerobic fermentation which was attributed to diffusion of the compounds into the brines and the formation of more stable compounds [318]. However, storing fresh unripe table olives in a CO_2 atmosphere resulted in elevated anthocyanin biosynthesis rates after 3 days of storage. This was different from the change in total phenolic and total flavonoid content [319]. Litchi fruit stored in air, MA and CA at 3°C generally exhibited decreasing anthocyanin content throughout storage with CA storage at low and high O_2 concentrations showing a slower downward trend compared to the MA treatment [296]. Thus, contrasting effects of MA and CA storage of fruit and vegetables are reported in the literature indicating that various plant matrices may behave differently even under the same or slightly modified storage conditions and that there is a need to assess further parameters to improve postharvest shelf-life.

Further treatments designed to improve postharvest quality during storage have been described. The coating of fresh fruit as a means of shelf-life extension has been studied using cold-stored litchi which show rapid quality loss due to surface browning when they are subsequently held at ambient temperature. Chitosan coating was effective in maintaining higher anthocyanin levels for an extended period of time and delaying pericarp browning [294]. In contrast, preharvest spraying of apples with aqueous kaolin suspensions has been applied to prevent sunburn of apples. A significant effect of the film on the fruit surface on anthocyanin content was not observed, *i.e.* treated apples were not different from control samples in terms of their surface colour [292]. Nowadays, MCP is commonly used as a postharvest inhibitor of ethylene synthesis which itself affects

anthocyanin accumulation. Whereas untreated apples showed a decrease in pigment content throughout storage, MCP treated fruit exhibited higher pigment levels and did not show a significant decrease after harvest [41].

Various parameters during juice processing affect the pigment stability and colour of the juices. Therefore, technologies for the preparation of products with high anthocyanin content and improved storage stability are required. Temperature strongly affects the anthocyanin content and stability. A systematic investigation of the temperature-time regimes applied for the hot water extraction of roselles revealed that these factors influence not only anthocyanin yields but also the colour of the extracts and the formation of polymeric pigments [317]. Elevated temperatures significantly enhance anthocyanin extraction by increasing diffusion coefficients, as was shown for pigment recovery in black carrots ranging from 25 to 50°C. Furthermore, yields of black carrot anthocyanins were also increased at pH 2.0 compared to pH 3.0 and 4.0 due to higher pigment stability in the acidic media [314]. Crushing, depectinization and centrifugation of fresh bayberries resulted in high pigment losses, additional blanching and pasteurization steps during processing yielded juices with significantly increased anthocyanin content. This can be attributed to PPO inactivation and increased cell permeation. However, up to 52–58% of the fresh fruit anthocyanins remained in the press residues. The percentage of polymeric anthocyanins and the browning index increased during the initial processing steps, whereas a gelatin–bentonite flocculation removed up to 94% of the polymeric and brown pigments [65].

High-temperature treatment (70°C, 30 min) of several grape cultivars for the production of grape juice revealed extraction rates of only 12–32% of the total anthocyanins present in the grapes. These results underline the need to optimize extraction methods to reduce the loss of these compounds [244]. As was shown for blueberries, the initial processing steps, such as thawing of frozen fruit, crushing, depectinization and pressing, usually contribute to large losses of anthocyanins, whereas clarification and concentration cause relatively little losses [58]. Mashing strawberries actually produced an increase in anthocyanin content, but this was attributed to incomplete extraction of the fresh fruit, and thus leads to underestimation of the amount of pigment in the raw material. Significant pigment losses occurred during both heating and fermentation of the mash and juice [276]. Even though the processing of strawberry juice concentrate and seedless purée generates only about 10 and 4% waste, the achenes from these waste products might be used as a source for the extraction of secondary metabolites, since they were shown to contain significant amounts of anthocyanins and further phenolic compounds [64].

Phenolic compounds may also be removed from fruit and vegetable juices by filtration and flocculation steps. However, only slightly reduced anthocyanin content was determined for pomegranate juice after ultrafiltration and preflocculation with gelatine and bentonite in combination with ultrafiltration when compared to control juices [283]. Nowadays, cell wall-degrading enzymes (mainly pectinases, cellulases and hemicellulases) are commonly applied during fruit and vegetable processing to enhance juice yields or for clarification. Usually, this also implies increased pigment extraction yields, but depending on side activities of the technical enzyme preparations anthocyanins may also be hydrolysed resulting in significantly decreased pigment levels and a different pigment profile of the juices or wines [280, 307]. In contrast, the pigment content of pomegranate juices was not affected when the juice was obtained either by peeling and centrifuge extraction or by squeezing the whole fruits, *i.e.* omitting thermal or enzyme-assisted steps [282].

Despite great efforts to maximize the recovery of polyphenols in general, and of anthocyanins in particular, in the course of fruit and vegetable processing (*e.g.* by enzymatic mash maceration, high-temperature short-time mash treatment and the application of pulsed electric fields), extraction remains incomplete [39, 58]. Thus, high anthocyanin content can be found in by-products of plant food processing. Anthocyanins are usually found in high quantities, or even exclusively, in the skins or outer parts of fruit and vegetables [13, 108, 180]. A screening of antioxidant activity and total phenolic and anthocyanin content in four apple cultivars revealed the values to be higher in the peels than in the flesh. Anthocyanins were exclusively found in the peel. Thus, the authors suggested exploiting apple peel by-products from apple sauce and canned apple manufacture for the production of functional food [217]. Statistical analyses showed that the antioxidative capacity is mainly attributed to flavan 3-ols in apple peel [114]. In red onions anthocyanins accumulated mainly in the dry skins and in the outer fleshy layer. These parts are usually removed by peeling prior to consumption. Consequently, only about 27% of the total anthocyanins of red onions are consumed [153]. Therefore, the by-products of vegetable processing, juice production and wine making are rich sources of anthocyanins. Both anthocyanin recovery and the pigment content of the press residues are significantly affected by juice processing techniques, such as the duration of skin contact, the degree of comminution, pressing techniques and fermentation [304]. For example, press residues from black currant processing were shown to contain anthocyanins as the major phenol class amounting to up to 95% of the total phenol content as determined by HPLC, thus contributing =74% to the radical scavenging activity of the pomace extracts [165].

Anthocyanins are increasingly utilized not only as natural colourants (E 163) substituting synthetic food additives, but also because of their biofunctional properties, which may be helpful in the prevention of certain degenerative diseases. Anthocyanins may either be recovered as crude extracts from the waste products of fruit and vegetable processing or

selectively purified and concentrated, *e.g.* by resin adsorption [288]. Resin adsorption and desorption with alcohols has proved to be a suitable method for increasing pigment concentration because of its high selectivity for phenolic compounds and because it does not change the pigment profile during processing [290]. Winery by-products have long been used for the production of enocyanin, which has been commercialized as a natural colourant since 1879. Besides anthocyanins these extracts contain a variety of other phenolic compounds, such as hydroxybenzoic and hydroxycinnamic acids, flavan 3-ols, flavonols and stilbenes [168]. Since high amounts of phenolic compounds are present in winery by-products, especially in the stalks, peel and seeds, extracts obtained from these parts exhibit antioxidant activity. Antioxidative properties were found to be correlated with total phenolic content. In contrast, a correlation between individual phenolic compounds and the antioxidant activity of the extracts could not be established [239]. This might be ascribed to the fact that only a few low-molecular compounds were quantified and that components such as oligomeric and polymeric compounds were not considered. Similar results were obtained for the antiradical activity of red and white grape pomace extracts. Differing activities, which could not always be correlated with total phenolic content, were attributed to differences in the composition of the phenolic fraction, such as the presence of anthocyanins in red grape pomace peel, not in the white, even though individual compounds were not quantified [263].

The anthocyanin profiles of processing waste from juice production may be different from those of fresh fruit, as has been shown for blueberry by-products [60]. This might be due to different stability characteristics of individual pigments during processing, *e.g.* as regards susceptibility towards enzymatic hydrolysis caused by side activities of technical enzyme preparations.

There are a growing number of papers dealing with the colour changes and stability of individual anthocyanins during vinification and storage of grape juice or red wines. The pigment profiles of grape skins and of the resulting wines after fermentation were found to differ. This could not be attributed to different extraction rates of individual compounds since the profile of the grape skins after maceration was still unchanged. It is more likely that the differences are due to enzymatic oxidation, polymerization or adsorption to yeast cell walls [258]. Systematic investigations revealed that maceration temperature has a higher impact on anthocyanin extraction than the duration of maceration. The latter was shown to have no effect on the quantitatively predominating compounds [267]. The pigment content of wine is not only significantly affected by the ripening stage of the grapes but also by the anthocyanin yields during maceration and fermentation, which, in turn, mainly depend on the ethanol content of the mash. Higher alcohol concentrations enhance the solubilization of anthocyanins, and other phenolic compounds, such as procyanidins [266]. A direct comparison of hot and cold pressing of grape mash revealed enhanced release of anthocyanins at elevated temperatures [122]. Significant differences in the anthocyanin content of wines were observed when carbonic maceration was applied rather than fermentation/maceration techniques using pumping-over or rotary vats. However, resulting wines with lower pigment amounts exhibited higher stability, and thus, after 2 years of storage, all wines showed comparable colour density values [270]. Cryomaceration before fermentation increased the pigment content, the colour density of wines and improved the colour stability. This is possibly due to the enhanced extraction of further phenolic compounds which may act as copigments [269].

The flash release process, which consists of rapid heating of the grapes and subsequent application of a strong vacuum, enhanced anthocyanin extraction. This method results in wines with significantly higher amounts of pigments compared with conventional winemaking techniques [54]. Macerating enzymes are increasingly used during winemaking, but enzyme preparations need to be thoroughly selected since differences in the enzyme activities and side effects of these technical preparations may not always be of advantage as regards the sensory characteristics of the resulting wines [323]. As an alternative to pectolytic enzyme preparations, yeast mutants producing a polygalacturonase have been investigated and shown to improve filterability of the musts and to increase anthocyanin content [260]. However, in a red grape model solution several yeast strains reduced anthocyanin content throughout fermentation. The loss of individual pigments was higher with increasing anthocyanin polarity. This loss was due to adsorption to the yeast cell wall, but also to the formation of novel coloured compounds [257].

During the aging process, the content of monomeric anthocyanins in wines decreases significantly [209]. Usually, a first-order kinetics rate is observed. However, this pigment loss accompanies the formation of numerous condensation and oxidation products changing the stability and colour attributes of the wines. Detailed statistical analyses have been performed to investigate the effects of individual compounds on colour stability [66, 173, 230, 241]. Delphinidin and petunidin 3,5-diglucosides were considerably less stable than the other anthocyanins in a Muscadine wine, retaining only 6–34% of their initial pigment content throughout storage at 20 and 37°C, respectively, for 60 days [2]. Therefore, individual pigments exhibit differing stability and reaction rates during wine aging. Due to the complex phenolic profile of grapes and young red wines a wide range of reactions may occur during winemaking and aging. The evidence suggesting that this might yield an even more complex composition of aged wines has recently been thoroughly reviewed by Monagas *et al*. [171]. Wine composition still remains largely unknown [207]. Recently, 129 different compounds have been identified and quantified in a red wine aged in oak barrels and glass bottles. These components belong to four pig-

ment families, the anthocyanins, pyranoanthocyanins, direct flavanol – anthocyanin condensation products and acetaldehyde-mediated flavanol – anthocyanin condensation products [268]. The mechanisms for the formation of such compounds, which cause the colour of red wine to change during aging from a bright red to a red brown tint, have been studied in detail in model systems [243]. Pyranoanthocyanins have also been detected in sparkling wines. Here, the second fermentation differed from the first with regard to the formation of novel pigments [246]. Although most studies on the change of the pigment profile during aging have been performed using wines and wine model systems, such pigments have also been detected in other plant extracts, *e.g.* anthocyanin – flavanol condensation products in black currant extracts [309].

The ripening stage of the grapes used for the production of aged wines is of particular importance since grapes harvested at an early stage exhibit lower pigment content, and this has been shown to affect the rate of pigment formation during aging [15]. After 2 years of storage in bottles, an average of 83 – 84% of monomeric anthocyanins in Tinta Miúda red wines were degraded, with cyanidin and malvidin glucosides showing the highest degradation rates. Novel compounds, such as pyruvic acid adducts, were formed [211]. The change in pigment profile throughout storage is most affected by the cultivation conditions of the grapes and the duration of aging, whereas other technological factors and the type of container (oak barrels or stainless steel tanks) have lower effects [259]. Sometimes, wine is aged in oak barrels or in steel tanks with added oak chips. The latter have been shown to decrease monomeric anthocyanins more rapidly than oak barrels, and also to produce higher colour intensities [248]. To investigate effects on colour and anthocyanin stability, the addition of copigments to grape juice has also been studied. A rosemary extract caused a bathochromic shift and hyperchromic effects but did not significantly alter the anthocyanin content. After pasteurization, pigment amounts were slightly lower than those in control juices [240]. Isoflavonoid extracts from red clover also improved the colour characteristics and pigment stability of grape juices and wines during short-time thermal treatment and a 9-wk storage period at 20 and 37°C [256]. The prefermentation addition of isolated rutin to grape mash enhanced copigmentation and anthocyanin extraction, whereas hydroxycinnamic acids caused converse results [172]. Co-winemaking of several grape cultivars was studied for the same purpose. This was shown to favour copigmentation and the enhanced formation of stable pigments during wine aging [52]. Detailed studies of these intermolecular copigmentation effects in model solutions have revealed that the stability is dependent on anthocyanin structure as well as on the nature of the copigment, or on the presence of further compounds, such as vitamin C [261, 327].

8.5 Summary: Anthocyanins

Several hundred anthocyanins have been identified. They can be found in a wide variety of plant foods. Studies on grapes and red wine predominate, but numerous other fruits, especially berries, and some vegetables have also been the subject of intense research activity. Most interestingly, the pigment content of fresh fruits may significantly increase throughout storage, even under cold storage conditions, due to ongoing biosynthesis of anthocyanins. Differing effects of MA and CA storage are reported in the literature, indicating that various plant matrices may behave differently even under the same storage conditions. Anthocyanins in processed foods are degraded during storage, with higher storage or process temperatures (blanching, pasteurization, drying) causing increased pigment loss. In the course of juice processing, heating steps may provide products with increased anthocyanin levels due to PPO inactivation and increased cell permeability and diffusion coefficients. Further steps involved in juice processing and winemaking, such as crushing of the fruits, depectinization, clarification and enzymatic mash treatment, may give rise to pigment loss. During storage, monomeric anthocyanins are degraded, but pigment loss accompanies the formation of numerous condensation and oxidation products changing the stability and colour attributes of the juices and wines. The novel compounds formed during winemaking and wine aging have been thoroughly studied, and complex reactions found. Wine composition still remains largely unknown. Despite great efforts to maximize anthocyanin recovery in the course of fruit and vegetable processing, extraction remains incomplete and produces by-products which are particularly rich in anthocyanins and might, therefore, be further exploited.

9 Conclusions

A lot of relevant sources of flavonoids are mentioned in the summaries of the sections: *e.g.* apples, apple juice, berries, broccoli and tomatoes for phenolic acids, *e.g.* apple, apple juice and tomatoes for chalcones, *e.g.* citrus fruits for flavanones, *e.g.* citrus fruits, grapes, lettuce and parsley for flavones, *e.g.* plums, apples, onions and blueberries for flavonols, *e.g.* tea, grapes, red wine and chocolate for monomeric flavanols and, *e.g.* grapes, red wine, berries and many other fruits and some vegetables for anthocyanins.

The expectation that the structural diversity within each subgroup, and the number of different procedures and parameters would make finding homogenous tendencies unlikely, has, in most instances, been confirmed. This means that generalizations based on the summaries of individual compounds classes can rarely be made, and considerations must be limited to the particular cases dealt with in the publications. By adding a database excel table combined with a

focused and unified evaluation, specific additional information has been rendered accessible and concise.

One thing that nearly all the cases examined have in common is that the effect of storage and technology-dependent food production on the polyphenol content is often negligible in comparison to the differences in content between different varieties of plants. Variety dependence must always be considered, for all classes of compounds.

The structural formulas are taken from the publication [1] of Prof. Rainer Cermak et al.

The authors have declared no conflict of interest.

10 References

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